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## Oviposition by small hive beetles elicits hygienic responses from Cape honeybees

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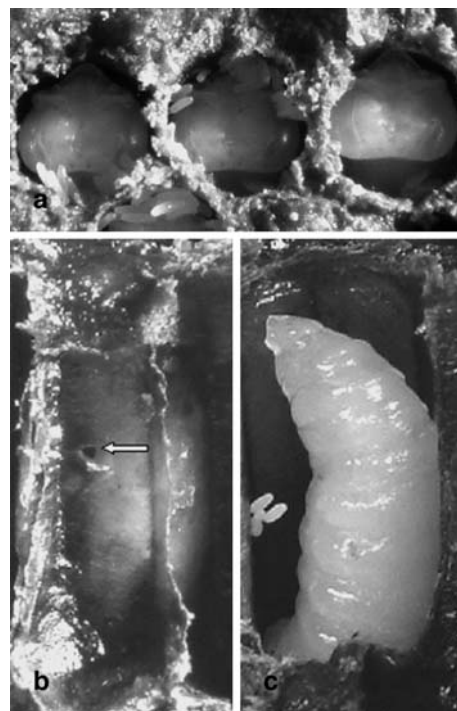
**Abstract** Two novel behaviours, both adaptations of small hive beetles (*Aethina tumida* Murray) and Cape honeybees (*Apis mellifera capensis* Esch.), are described. Beetles puncture the sides of empty cells and oviposit under the pupae in adjoining cells. However, bees detect this ruse and remove infested brood (hygienic behaviour), even under such well-disguised conditions. Indeed, bees removed 91% of treatment brood (brood cells with punctured walls caused by beetles) but only 2% of control brood (brood not exposed to beetles). Only 91% of treatment brood actually contained beetle eggs; the data therefore suggest that bees remove only that brood containing beetle eggs and leave uninfested brood alone, even if beetles have accessed (but not oviposited on) the brood. Although this unique oviposition strategy by beetles appears both elusive and adaptive, Cape honeybees are able to detect and remove virtually all of the infested brood.

### Introduction

Resistance of African honeybees (*Apis mellifera* L.) to small hive beetle (*Aethina tumida* Murray) depredation is partially due to beetle imprisonment that precludes access to brood, honey, and pollen reserves in the combs (Neumann et al. 2001) where its reproductive potential is very high (Ellis et al. 2002). Although confining beetles was thought to be unique to their natural honeybee hosts in Africa, this behaviour also occurs in otherwise beetle-naïve, European-derived honeybees in North America

(Ellis et al. 2003c, 2003d), which are often extremely susceptible to beetle depredation (Hood 2000; Ellis et al. 2003b). Therefore, the confinement of beetles cannot be the sole reason African honeybees are immune to beetles while European bees are not.

If female beetles reach the brood combs, they may puncture the waxy capping of brood cells and lay eggs on and around the honeybee pupa (Fig. 1a; Ellis et al. 2003a). On hatching, beetle larvae feed on the brood and severely



**Fig. 1a–c** Small hive beetle oviposition through cell cappings (mode 1) and walls (mode 2). **a** Oviposition directly through cell cappings (removed; Ellis et al. 2003a). Beetles also puncture cell walls (arrowed in **b**). When the cell wall is removed, beetle eggs are seen around the honeybee pre-pupa (**c**). Alternatively, the punctures may be made closer to the bottom of the cell and the eggs laid under the pupa. Photograph by James Greaves

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damage colonies of European honeybees (Hood 2000). Nonetheless, honeybees generally show hygienic responses to other pests and diseases and remove infected brood (Rothenbuhler 1964a; cf. Boecking and Spivak 1999). We therefore tested for the expression of hygienic behaviour by Cape honeybees (*A. m. capensis* Esch.) towards beetle eggs oviposited in bee brood.

## Methods

Experiments were conducted at an apiary near Grahamstown, South Africa in April 2003. Ten hived colonies of Cape honeybees of equal strength and reserves were used. All colonies had existing beetle populations (<50 beetles). For each colony, a frame of capped brood was removed and 20 randomly collected adult beetles were placed on a 10×10-cm area on the frame (treatment) in a sheet-metal push-in cage (10×10×2.5 cm, L×W×H), the face of which was screen mesh to allow for ventilation. The combs used contained about 50% empty and 50% capped brood cells. A second cage without beetles was pushed into the brood frame as a control. Both caged sections of brood were placed in the center of the bee cluster in each colony.

Twenty-four hours later, both cages were removed and the adult beetles from the treatment cage were collected. Beetle oviposition punctures in the capped cells were noted. Previous work showed that beetles puncture brood cell cappings (Fig. 1a; Ellis et al. 2003a); however, in this study, we observed puncture marks well down the sides of capped cells (Fig. 1b). A transparent sheet of plastic was placed on the comb and all capped brood with punctures in the cell walls were marked. Similarly, 20 uninfected capped brood cells from the control cage were marked. The treated and control brood were replaced in the center of the bee cluster. After 48 h they were examined and marked cells from which infected or control brood had been subsequently removed by the bees were counted (Table 1).

The infestation rate of treatment cells containing punctures made by beetles was determined (Table 1). For each of seven colonies, 20 adult beetles were confined to one frame of capped brood as before and the frames were replaced in the colonies. Twenty-four hours later about 30 cells from each frame having punctures in their walls were opened to determine the presence/absence of beetle eggs. These data were used to determine the infestation rate of brood cells containing punctures. The number of beetle eggs per infested cell was also determined.

Differences in the proportion of removed brood were analyzed by independent sample *t*-tests recognizing brood condition (control brood or treatment brood with oviposition punctures) as the main

effect. Because of the analysis of proportions of removed brood, the data were transformed using  $\arcsin\sqrt{\text{proportion}}$  to stabilize the variance. Likewise, the proportion of removed treatment brood was tested for differences from the proportion of infested brood (proportion of cells with punctures and containing beetle eggs) using independent sample *t*-tests and  $\arcsin\sqrt{\text{proportion}}$  transformations as before. All differences were accepted at  $\alpha \leq 0.05$ .

## Results

Previously, only one mode of beetle oviposition on brood was known: oviposition directly through cell cappings (mode 1, Fig. 1a; Ellis et al. 2003a). In this study, we also found that beetles enter and puncture the walls of empty cells then oviposit in adjacent cells containing capped brood (mode 2, Fig. 1b, c). Sometimes, the eggs were laid under the pupa and could only be detected by removing the pupa. In other instances, the punctures were midway down the cell wall and the eggs were laid around the pupa (Fig. 1b, c). The proportion of treatment cells (having punctures) infested with beetle eggs was  $0.905 \pm 0.024$  (mean  $\pm$  SE,  $n=7$  colonies; individual colony data are reported in Table 1). Further, 168 infested cells in seven colonies contained an average of  $33.9 \pm 1.8$  beetle eggs per cell (totalling approximately 5,695 eggs for the seven colonies or about 814 eggs/colony).

Brood condition (treatment or control) significantly affected the proportion of brood removed by the bees ( $|t|=18.94$ ;  $df=18$ ;  $P<0.0001$ ). The proportion of treatment brood removed by the bees ( $0.907 \pm 0.024$ ; mean  $\pm$  SE,  $n=10$  colonies) was higher than the proportion of control cells removed by the bees ( $0.017 \pm 0.011$ ; mean  $\pm$  SE,  $n=10$  colonies; individual colony data are reported in Table 1). Indeed, only two colonies removed control cells leading to the mean reported above (the first colony removed two and the second removed one); no other colonies removed any of the control brood.

Additionally, there was no significant difference between the proportion of treatment brood removed by the bees and the proportion of treatment brood infested with beetle eggs ( $|t|=0.14$ ;  $df=15$ ;  $P=0.8913$ ). Therefore, it is reasonable to conclude that although bees were only

**Table 1** Data on treatment and control brood removal by Cape colonies and on the infection rate of cells containing punctures (punctured cells containing beetle eggs)

Colony	Treatment		Control		Infection rate <sup>a</sup>	
	No. cells with punctures	No. cells removed	No. marked cells	No. cells removed	No. cells with punctures	No. cells containing eggs
1	29	26	20	0	22	20
2	30	22	20	0	30	25
3	16	15	20	0	30	30
4	19	17	20	0	14	13
5	79	69	15	1	30	26
6	12	12	20	0	30	25
7	10	10	20	0	30	29
8	21	20	20	0	n.a.	n.a.
9	16	14	20	2	n.a.	n.a.
10	21	19	20	0	n.a.	n.a.

<sup>a</sup> Only seven colonies were tested for infection rates so data for colonies 8–10 were not collected and are therefore not available (n.a.)

removing 91% of all treatment brood, they were removing all of the brood actually infested with beetle eggs (which was also 91%). We validated this assumption by opening treatment brood cells not removed by the bees and in no case were beetle eggs found.

## Discussion

Lundie (1940) and Schmolke (1974) found that beetles oviposit in cracks in the hive around the nest periphery and directly in pollen cells. However, these modes of oviposition appear to contribute little to the overall reproductive potential of the beetles as larvae hatching from eggs in the nest periphery have to crawl to the combs, and studies have shown that African subspecies of honey bees very rapidly remove free-roaming beetle larvae from the colony (Neumann and Härtel 2003). Further, at low beetle populations, most beetles are confined to the nest periphery and oviposition has never been observed during beetle confinement (Ellis et al. 2003c, 2003d). Therefore, beetle oviposition directly into bee brood is more likely to result in scores of unnoticed larvae than is beetle oviposition in cracks and crevices around the nest.

Ellis et al. (2003a) described beetles puncturing cell cappings and laying eggs directly on bee pupae in European bee colonies when bees were present (mode 1). Beetles would normally have little chance to oviposit through cell cappings in African colonies as African bees display high aggression towards free-running beetles (Elzen et al. 2001). However, beetles are often found in empty cells among the combs (Ellis 2003c, 2003d) where they retreat to the bottom of the cell, exposing only their hard exoskeleton to any bee aggression. Such beetles would then be able to oviposit in adjacent cells containing brood and successfully reproduce (mode 2). These oviposition tactics by the beetles to conceal their eggs appear inevitably foiled because Cape bees removed virtually all infested brood.

Although how bees detect infestation/infection in capped brood is not known (cf. Boecking and Spivak 1999), pathogen-killed brood may be easily recognized and removed by the bees (Rothenbuhler 1964a). However, while pests such as varroa mites (*Varroa destructor* Anderson and Trueman) do not necessarily kill brood, the bees are able to detect and remove the brood nonetheless. There are strong indications that bees cue into the presence of beetle eggs and not the punctures created by the beetles as no brood was removed from punctured cells not containing beetle eggs. Further, Neumann and Härtel (2003) have shown that unprotected eggs in a colony are removed within 24 h. If beetle eggs stimulate brood removal by bees, our study does not determine the number of eggs/cell necessary to elicit hygienic responses from the bees because cells in this study contained a large number of beetle eggs. Therefore, there may exist a minimum number of eggs/cell that elicits brood removal.

Even though Cape bees remove beetle eggs from capped brood (present study) and free-roaming larvae from the colony (Neumann and Härtel 2003), thus minimizing beetle reproduction, beetles maintain a continued presence in Cape bee colonies. This further implies that beetle reproduction in their native range is limited to weakened/diseased colonies (Lundie 1940) or nests left by absconding bees (Hepburn and Radloff 1998) because of behavioural responses of their honeybee hosts.

To place this study in a wider context it must be remembered that Rothenbuhler (1964b) proposed a two-gene model to explain phenotypic variance in hygienic behaviour, suggesting that one locus controls the uncapping of brood cells and the second controls removal of the cell contents. However it has recently been suggested (Moritz 1988; Lapidge et al. 2002) that more than two loci are responsible for hygienic behaviour, implying that hygienic behaviour is more complex than the uncapping and subsequent removal of diseased/infested brood. Our data support the view that hygienic behaviour may be more complex than once thought because Cape bees remove only that brood containing beetle eggs, thus exercising discriminative and selective removal of infected brood only.

Although a suite of behavioural/environmental factors are probably responsible for overall Cape bee resistance to beetles, our data clearly show that Cape honeybees can detect and remove brood infested with beetle eggs. Hygienic behaviour likely contributes to Cape bees' success in thwarting potential damage caused by beetles. Indeed, that we found over 33 beetle eggs/infested cell suggests that had the bees not removed the infested brood, the colonies would have been quickly overrun by beetle larvae, as occurs among European-derived honeybees in North America.

Related kinds of hygienic behaviour towards other pathogens already exist in European bees (cf. Boecking and Spivak 1999; Spivak and Boecking 2001), and preliminary data suggest that hygienic behaviour towards beetle eggs is also present in European colonies (but is less pronounced than in African colonies, unpublished data). Therefore, resistance to beetles by European colonies may be improved because the behaviour is amenable to selective breeding programs (Harbo and Harris 1999). In conclusion, the data suggest that hygienic behaviour does not target any one brood-infecting pathogen but is instead a more general response to a suite of brood conditions that may ultimately weaken or destroy a colony.

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## References

- Boecking O, Spivak M (1999) Behavioral defenses of honey bees against *Varroa jacobsoni* Oud. *Apidologie* 30:141–158
- Ellis JD Jr, Neumann P, Hepburn HR, Elzen PJ (2002) Longevity and reproductive success of *Aethina tumida* (Coleoptera: Nitidulidae) fed different natural diets. *J Econ Entomol* 95:902–907
- Ellis JD Jr, Hepburn HR, Delaplane KS, Elzen PJ (2003a) A scientific note on small hive beetle (*Aethina tumida*) oviposition and behavior during European (*Apis mellifera*) honey bee clustering and absconding events. *J Apic Res* 42:47–48
- Ellis JD Jr, Hepburn HR, Delaplane KS, Neumann P, Elzen PJ (2003b) The effects of adult small hive beetles *Aethina tumida* (Coleoptera: Nitidulidae), on nests and flight activity of Cape and European honey bees (*Apis mellifera*). *Apidologie* 34:399–408
- Ellis JD Jr, Hepburn HR, Ellis AM, Elzen PJ (2003c) Prison construction and guarding behavior by European honeybees is dependent on inmate small hive beetle density. *Naturwissenschaften* 90:382–384
- Ellis JD Jr, Hepburn HR, Ellis AM, Elzen PJ (2003d) Social encapsulation of the small hive beetle (*Aethina tumida* Murray) by European honeybees (*Apis mellifera* L.). *Insectes Soc* 50:286–291
- Elzen PJ, Baxter JR, Neumann P, Solbrig A, Pirk CWW, Hepburn HR, Westervelt D, Randall C (2001) Behavior of African and European subspecies of *Apis mellifera* toward the small hive beetle, *Aethina tumida*. *J Apic Res* 40:40–41
- Harbo JR, Harris JW (1999) Selecting honey bees for resistance to *Varroa jacobsoni*. *Apidologie* 30:183–196
- Hepburn HR, Radloff S (1998) Honeybees of Africa. Springer, Berlin Heidelberg New York
- Hood WM (2000) Overview of the small hive beetle, *Aethina tumida*, in North America. *Bee World* 81:129–137
- Lapidge KL, Oldroyd BP, Spivak M (2002) Seven suggestive quantitative trait loci influence hygienic behaviour of honey bees. *Naturwissenschaften* 89:565–568
- Lundie AE (1940) The small hive beetle, *Aethina tumida*. Union of South Africa, Department of Agriculture and Forestry, Science Bulletin 220
- Moritz RFA (1988) A reevaluation of the two-locus model hygienic behaviour in honey bees, *Apis mellifera* L. *J Hered* 79:257–262
- Neumann P, Härtel S (2003) Removal of small hive beetle (*Aethina tumida* Murray) eggs and larvae by African honeybee colonies (*Apis mellifera scutellata* Lepeletier). *Apidologie* 34 (in press)
- Neumann P, Pirk CWW, Hepburn HR, Solbrig AJ, Ratnieks FLW, Elzen PJ, Baxter JR (2001) Social encapsulation of beetle parasites by Cape honeybee colonies (*Apis mellifera capensis* Esch.). *Naturwissenschaften* 88:214–216
- Rothenbuhler WC (1964a) Behavior genetics of nest cleaning behavior in honeybees I. Response of four inbred lines to disease killed brood. *Anim Behav* 12:578–583
- Rothenbuhler WC (1964b) Behavior genetics of nest cleaning in honey bees IV. Responses of F<sub>1</sub> and backcross generations to diseased-killed brood. *Am Zool* 12:578–583
- Schmolke MD (1974) A study of *Aethina tumida*: the small hive beetle. Certificate in Field Ecology Project Report. University of Rhodesia (Zimbabwe), Salisbury (Harare)
- Spivak M, Boecking O (2001) Honey bee resistance to varroa mites. In: Webster TC, Delaplane KS (eds) *Mites of the honey bee*. Dadant, Hamilton, Ill., pp 205–227